Summary

Keratoconus (KTCN) is a progressive degenerative disease of the cornea that affects the general population with a frequency of 1 in 2,000 people. KTCN is characterized by increasing thinning of the cornea, resulting in its conical shape, which leads to significant visual impairment. The first symptoms of the disease may appear in adolescence and then progress through the third/fourth decade of life. A higher occurrence of the KTCN in male patients has been reported.

KTCN is considered a multifactorial disease, in which a complex genetic background embraces numerous identified chromosomal *loci*, including 13q32, 5q31.1-q35.3, 2q13-q14.3, 20p13-p12.2, and sequence variants in candidate genes, such as *VSX1*, *DOCK9*, *STK24*, *IPO5,* and *TGFBI*. Some environmental factors have been previously reported as causative in KTCN etiology. Historically, KTCN was considered a non-inflammatory disease, however, this aspect is now questioned by clinicians and researchers due to the reports indicating the elevated expression of cytokines and proteases in patients' tear fluid and serum samples.

In patients with early and moderate KTCN, the cornea evaluated by slit lamp biomicroscopy may appear normal. In order to diagnose KTCN, a patient should undergo a comprehensive ophthalmological examination, including assessment of uncorrected and corrected visual acuity, slit lamp examination of the anterior segment of the eye, corneal tomography (Scheimpflug camera), and anterior segment tomography (AS-OCT).

Histopathological abnormalities of the cornea of patients diagnosed with KTCN comprise all corneal layers. The most characteristic histological change is the thinning of the corneal stroma. In turn, the corneal epithelium (CE), trying to 'mask' the emerging stromal irregularities, becomes the thinnest at the top of the cone and forms a characteristic *doughnut pattern*.

In this doctoral dissertation, it was hypothesized that the remodeling of the corneal epithelium in the course of KTCN is an important element of the clinical picture of the disease. Previously the characteristic alternations in the thickness of the corneal epithelium, called *doughnut pattern*, have not been considered in the molecular studies. Therefore, attempts to interpret the integrated clinical and molecular findings biologically have been insufficient.

This doctoral dissertation is a collection of four publications resulting from experimental studies of transcriptomic, proteomic, coding, and non-coding genome features of the corneal epithelium, epidemiological studies identifying environmental factors determining the KTCN phenotype, and a literature review.

Based on previous KTCN studies, covering mainly selected molecular and/or clinical aspects, as well as the results of own research, it was concluded that solely multi-level molecular approach and integration of different scientific fields provide the opportunity for a deeper understanding of the biological aspects in the complex nature of KTCN.

The conducted research took into account the diversity of morphological features in the cornea, defining for the first time three regions within the corneal epithelium and introducing a new definition of the topographic regions. Based on abnormalities in CE thickness profiles in patients with KTCN, the three topographic regions were characterized (central, middle, and peripheral) with specific morphological, transcriptomic, and proteomic alternations. As a result of the integration analyses, a mechanism of corneal epithelium remodeling was proposed,

highlighting the dysregulation of epithelial-mesenchymal transition, cell-cell communication, and cell-extracellular matrix interactions pathways, contributing to the wound healing process, which indirectly influences the formation of the keratoconic cone. These recognized alternations allow to conclude that the presence of three topographic corneal regions should be taken into account during the experimental study design and biological interpretation of the molecular findings.

The previous assumption regarding chronic mechanical corneal injury in KTCN was verified. The eye rubbing, not correlated with an allergy, causes CE damage and triggers cellular stress, which, through its effect on apoptosis, migration, and cell adhesion, also influences the KTCN phenotype.

During this study the whole genome sequencing (WGS) technique, which has not been used in the worldwide research on KTCN so far, was utilized. The multiple coding and non-coding sequence variants were found in genes or in their close proximity, contributing to innate and adaptive immune system responses, which indicates a more complex inflammatory background in KTCN than previously anticipated. Moreover, 35 chromosomal *loci* were identified as significantly enriched in recognized coding and non-coding KTCN-specific sequence variants, including 5q, 9q, and 16q *loci*, previously recognized as etiologically related to KTCN.

The conducted studies confirmed the complexity of the genetic/molecular background of KTCN and the importance of recurrent/persistent inflammation (which may have a genetic basis) on the clinical picture of the keratoconus.